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PHYSIOLOGICAL ROLES OF PROSTAGLANDINS AND OTHER EICOSANOIDS IN INVERTEBRATES

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Abstract

Prostaglandins and other biologically active derivatives of polyunsaturated fatty acids have been detected in a large number of invertebrate species. A brief summary of the mammalian background of arachidonic acid metabolism is provided, and the physiological significance of these compounds in invertebrates is reviewed. Topics include regulation of ion flux, temperature regulation, reproductive biology, cell aggregation, and host-parasite interactions. Finally, perspectives on current and possible future research are offered.

Introduction

The term eicosanoid was introduced and used by Corey *et al.* (1980) to describe the various biologically active derivatives of eicosapolyenoic fatty acids, especially arachidonic acid. So far, we know of four major groups of eicosanoids: the prostaglandins (PGs), the hydroperoxy- and hydroxyeicosatetraenoic acids (HPETEs and HETEs), the leukotrienes (LTs), and the lipoxins (LXs). Interest in the significance of eicosanoids in the biology of mammals stems from physiological studies conducted in the early twentieth century. In the earliest reference to one group of eicosanoids, the PGs, Jappelli and Scafa (1906) noted that extracts of dog prostrate glands caused paralysis of central respiratory control and changed heart rates when injected into dogs and rabbits. The discovery of PG pharmacological activity in human seminal fluids (Kurzrok and Lieb, 1930) probably marks the beginning of the detailed studies of the clinical significance of these compounds. Elucidation of the chemical structures of PGs in the early 1960's (Bergström *et al.*, 1962a, b) greatly increased the pace of research and discovery, hindered in that decade mainly by the limited availability of working quantities of purified compounds. It is now known that PGs are present and play important roles in almost all mammalian tissues and fluids (Horrobin, 1978). Examples of PG action include pathophysiological actions such as mediation of the inflammatory response (which we commonly block by ingestion of aspirin) and participation in the blood-clotting cascade, as well as physiological actions such as contraction of smooth muscle.

The growth of PG research began with initial physiological observations, along with isolation and structural determinations of individual PGs. This was followed by the development of techniques to produce PGs in a commercially profitable way for clinical and biological studies. Commercial production of PGs evolved from biosynthesis from appropriate precursor fatty acids using large-scale enzyme preparations, through the discovery of naturally occurring sources of PGs and of intermediates in chemical synthesis to economical total synthesis. Hence, the first report of PGs in

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Abbreviations: PG = prostaglandin, LT = leukotriene, HETE = hydroxyeicosatetraenoic acid, HPETE = hydroperoxyeicosatetraenoic acid, LX = lipoxin.

an invertebrate animal, the gorgonian coral *Plexaura homomella* (Weinheimer and Spraggins, 1969), met with tremendous interest, not as a zoological discovery, but as a commercial source of PG for laboratory study. In the years between this first discovery of a potentially economical source of PG and the development of appropriate synthetic strategies, the search for other biological sources of PGs turned up many examples of their occurrence in marine invertebrates, albeit at tissue concentrations far below the point of commercial interest.

One of the PGs in greatest abundance in the coral, 15-epi-PGA_2, is not pharmacologically active in the usual mammalian biological assays for PG activity (Nakano, 1969). Chemical modification of the naturally occurring form to clinically useful structures, as well as commercial, ecological, and environmental aspects of sustained PG yield from coral have been reviewed (Theoder, 1977; Berte, 1981; Bundy, 1985). Other papers describe evidence for the occurrence of PGs in over one hundred invertebrate species. Christ and van Dorp (1972) detected PG-biosynthesis activity in five invertebrates—including two coelenterates, a mollusc, an annelid and an arthropod—but not in two insect species. Using a classical bioassay for the pharmacological effect of PG on contraction of mammalian smooth muscle, Nomura and Ogata (1976) detected PGs in a procordate, and in representatives of Echinodermata, Mollusca, Annelida, Coelenterata, and Arthropoda (including an insect). PGs were also detected by bioassay in saliva of another terrestrial arthropod, the tick *Boophilus microplus* (Dickinson *et al.*, 1976; Higgs *et al.*, 1976). Using radioimmunoassay, Shemesh *et al.* (1979) found PGs in reproductive organs and salivary glands of another tick. Since all PGs are formed from a common intermediate, prostaglandin-endoperoxide, PG synthesis could be inferred from activity of prostaglandin-endoperoxide synthetase. Morse *et al.* (1978) detected this enzyme activity in 41 species of coelenterates collected in the Caribbean Sea and the Pacific Ocean. Gromov *et al.* (1982) used radioimmunoassay to estimate amounts of two PGs in a snail. Korotchenko *et al.* (1983) found smooth muscle-contracting activity in 10 echinoderm species; they also refer to finding PG activity in 40 other invertebrates.

Aside from detection of PGs in a large number of invertebrate species, certain reports suggest that eicosanoids play fundamental physiological roles in representatives of many invertebrate phyla. Such findings are interesting because they provide insights into the details of regulatory physiology. Interest extends to an evolutionary axis because discovery of eicosanoid physiology especially in the very early phyla suggests that the significance of these compounds is not limited to vertebrate and clinical physiology, but was established early in metazoan evolution.

Evolutionary interest may eventually extend to plants, as well. Gregson *et al.* (1979) described the occurrence of two PGs in the red alga *Gracilaria lichenoides,* and Janistyn (1982) reported chemical identification of $PGF_{2\alpha}$ in the flowering plant *Kalanchoe blossfeldiana.* A prostaglandin-like compound was produced from linolenic acid by an enzyme preparation of flaxseed (Zimmerman and Feng, 1978). The physiological significance of these compounds in plants is not clear, but compounds that inhibit PG-biosynthesis in mammals inhibited growth in four fungus species (Herman and Herman, 1985; Kerwin *et al.*, 1986). Earlier inhibitor studies showed inhibition of flowering in *Pharbitis nil* (Groenewald and Visser, 1974). Although these findings are preliminary, they suggest that eicosanoids may be of broad biological significance.

The goal of this review is to provide an appreciation of the physiological significance of eicosanoids in invertebrate animals. Since the appropriate nomenclature and physiological background comes from decades of work on various mammal systems, it is useful to begin with a background from mammal studies.

A Background from Mammalian Studies

Upon stimulation by various agonists, many mammal cells hydrolyze polyunsaturated fatty acids (PUFAs), by action of phospholipase A2, from the beta carbon of membrane phospholipids. Three C20 PUFAs—dihomo-γ-linolenic (C20:3n6), arachidonic (C20:4n6), and eicosapentaenoic (C20:5n3) acids—may be metabolized by one of two major pathways into biologically active molecules. In the cyclooxygenase pathway, PUFAs are transformed into prostaglandins and thromboxanes, whereas the lipoxygenase pathway leads to hydroperoxy- and hydroxypolyenoic fatty acids which are themselves biologically active as well as further metabolized into lipoxins and leukotrienes. Since these are all derivatives of C20 PUFAs, they may be collectively referred to as eicosanoids. The following description of the biosynthesis and physiological roles of these compounds in mammals is assembled from several reviews and books (Horrobin, 1978; Samuelsson *et al.*, 1978; Hansson *et al.*, 1983; Samuelsson, 1983; Serhan *et al.*, 1985), and is presented with minimum referencing.

PGs are C20 carboxylic acids with a five-membered ring variously substituted at C9 and C11, and two aliphatic chains featuring a substitution at C15 and one, two, or three double bonds. The structures of the principle PGs are shown in Figure 1. PGs are designated as lettered and numbered series. The numbers indicate the number of aliphatic double bonds, giving rise to the one-, two-, and three-series PGs. The letters are associated with the particular pattern of substitutions on the five-membered ring: PGE features C9 keto, C11 hydroxyl substitutions; PGF a C9,C11 dihydroxyl pattern; PGD a C9 hydroxyl, C11 keto arrangement. PGs of the A, B, D, E, and F series are so distinguished.

Biosynthesis of PGs is a multistep operation beginning with formation of the prostaglandin endoperoxides—first PGG—by action of microsomal prostaglandin endoperoxide synthetase. The same enzyme also cleaves the hydroperoxy group of PGG to form PGH. PGH is the root intermediate in the synthesis of the primary PGs: PGD is formed by a glutathione-S-transferase, PGE requires prostaglandin endoperoxide E isomerase and PGF prostaglandin endoperoxide reductase; PGI is formed by prostaglandin endoperoxide I isomerase and thromboxane A (TxA) by prostaglandin endoperoxide thromboxane A isomerase.

PGs have been detected in most mammalian tissue systems where they are involved in many well-catalogued (Horrobin, 1978) physiological activities. Examples of PG action include contraction of smooth muscle (*i.e.*, uterine, gut, and blood vessel), attenuating cellular response to hormones, and release of digestive acid in the stomach. Thromboxane A_2 is a potent inducer of platelet aggregation; its name is taken from its origin, the thrombocytes.

Lipoxygenase pathways first lead to hyproperoxy fatty acids which can be reduced by peroxidases, and possibly by non-enzymatic reactions, to corresponding hydroxy fatty acids (Fig. 2). Arachidonic acid is the best-studied lipoxygenase substrate in mammals, and oxygen can be added at various positions, leading to 5-, 8-, 9-, 11-, 12-, and 15-hydroxyeicosatetraenoic acids (the various HETEs). Di- and tri-hydroxy fatty acids also can be formed by lipoxygenase acting on the same fatty acid substrate more than once; another route to trihydroxy acids is by way of an epoxy-hydroxy acid. While PGs are involved in various physiological as well as pathophysiological actions, the lipoxygenase products apparently are involved in pathophysiological actions such as bronchial constriction. The lipoxygenase reactions are found in defense systems such as the various leukocytes, macrophages, monocytes, lung, and spleen. HETEs are biologically active in defense roles. For example, 5-, 9-, and 11-HETE are all active in inducing the chemokinesis and chemotaxis associated with migration of eosinophils into the site of certain hypersensitivity reactions.

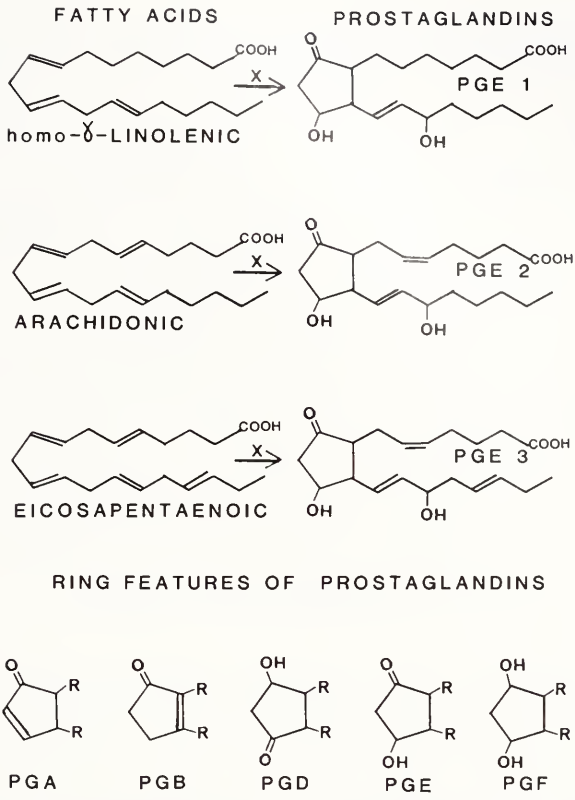

FIGURE 1. Relationship between the 1-, 2-, and 3-series prostaglandins and their parental polyunsaturated fatty acids, respectively C20:3n6, C20:4n6, and C20:5n3, is indicated by the arrows. × indicates cyclooxygenase activity. Ring features of five prostaglandins are shown in the lower panel where R stands for the aliphatic chains shown on the complete structures.

The leukotrienes (LTs; Fig. 3) were discovered during work on rabbit polymorphonuclear leukocytes, and take their names from this and the conjugated triene structure they have in common. The following description of LTs comes from the review by Samuelsson (1983). There are two classes of leukotrienes: the cysteine-containing group (LTC_4, LTD_4, and LTE_4), and LTB_4, which is not substituted. Biosynthesis of the LTs begins with formation of 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) by action of lipoxygenase followed by conversion to LTA_4 by abstraction of a hydrogen and elimination of a hydroxyl anion, catalyzed by a soluble enzyme, dehydrase. LTA_4 is converted to LTB_4 by hydrolase, or into the parental cysteine-containing LT (LTC_4) by a glutathione-S-transferase. The cysteine-containing LTs feature a thioether linkage at C6 to cysteine; LTC_4 is γ-glutamyl-cysteinyl-glycyl substituted; glutamyl transpeptidase elimination of the glutamine residue forms cysteinylglycyl LTD_4 which can be metabolized into cysteinyl LTE_4.

LTs have been identified in several cell systems including rabbit, human, mouse, and rat leukocytes; mouse and rat macrophages; and human and guinea pig lung. The biological significance of these compounds lies in their identification as the slow-reacting substance of anaphylaxis (SRS-A). This material is a mediator in asthma and other mammalian hypersensitivity reactions; SRS-A is released with other mediators

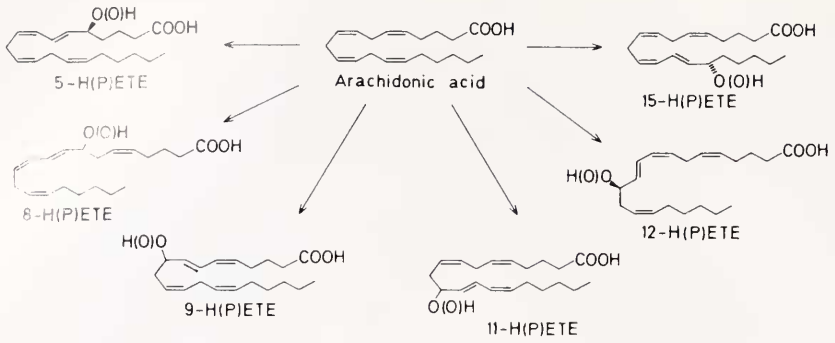

FIGURE 2. Structures of lipoxygenase metabolites of arachidonic acid, hydroxyeicosatetraenoic and hydroperoxyeicosatetraenoic acids.

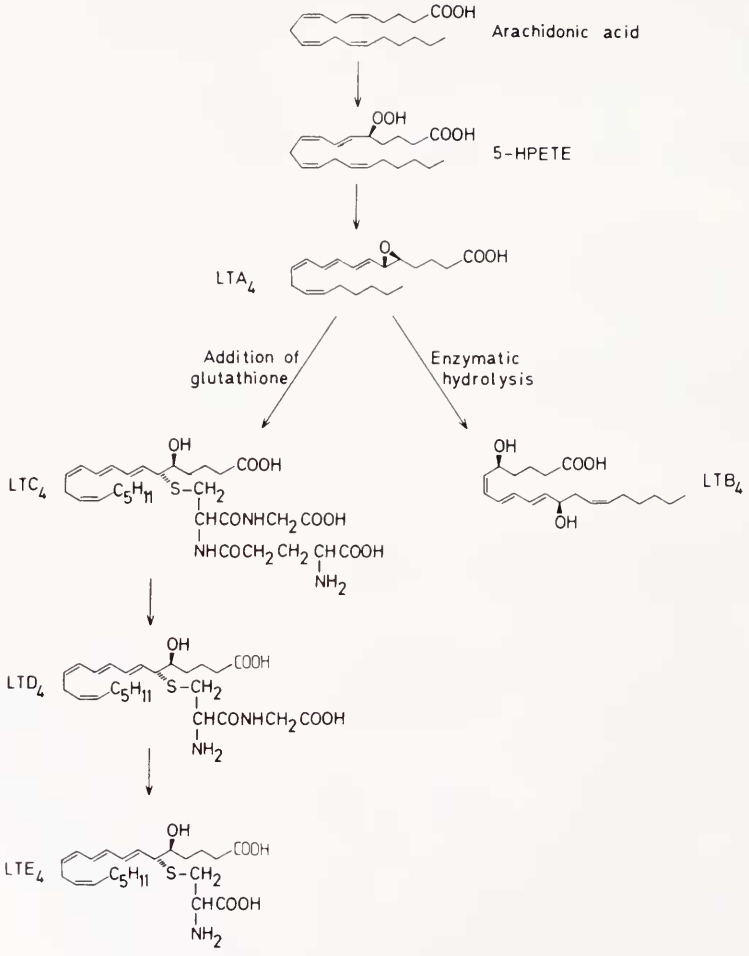

FIGURE 3. Structures of leukotrienes.

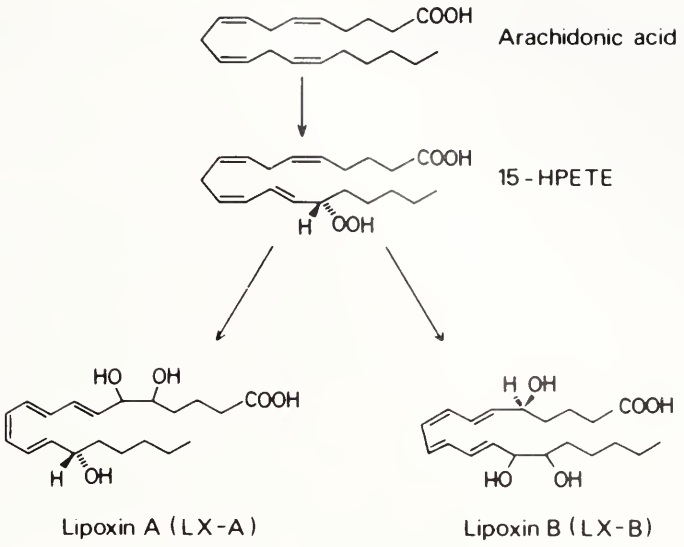

FIGURE 4. Structures of lipoxins.

after interaction of antigens such as pollen with immunoglobulin. SRS-A is a mixture of the cysteine-containing LTs. LTB_4, which does not contain cysteine, stimulates enzyme release, adhesion of neutrophils to endothelial cells, and movement of fluids through vessel walls in microcirculation. Lindgren *et al.* (1985) showed that LTs occur in the rat brain—most prominently in the hypothalamus and median eminence—and that they may be involved in hormone release by brain cells.

The lipoxins (Lx; Fig. 4) are the most recently discovered metabolites of arachidonic acid. They share the characteristic feature of a conjugated tetraene structure. Two major LXs, LXA and LXB, were formed by human leukocytes; LXA stimulated oxygen metabolism and generation of active oxygen species in human neutrophils. The action of LXA in neutrophils differs from the action of leukotriene B_4 and may represent another physiological mechanism of host defense. Lipoxins appear to be formed by 5-lipoxygenase activity on a substrate formed by 15-lipoxygenase metabolism of arachidonic acid. (The trivial name lipoxins is an abbreviation of lipoxygenase interaction products.)

The PGs, LTs, and LXs are involved in basic physiological processes at the cellular level and appear to be especially important in various pathophysiological responses such as inflammation, blood-clotting, asthma, and tumor growth. Due to their clinical significance, much effort is directed toward appreciating the regulation of arachidonic acid metabolism and developing specific inhibitors of PG, LT, and LX biosynthesis. Specific compounds will be mentioned in the contexts of biological studies in various invertebrate systems. Here it should be mentioned that within a given mammalian system there is considerable tissue variation in the effects of various inhibitors; moreover, there is variation between mammalian species. In light of tissue and specific variations in cyclooxygenase and lipoxygenase systems in mammals, one notes that the considerable literature on mammals should not be taken as a set of rules of the biochemistry of fatty acids in invertebrates. It is more appropriate to interpret the background as a loose set of guidelines, likely to be misleading at

crucial points in our consideration of the physiological significance of eicosanoids in invertebrates.

Physiological Significance of Eicosanoids in Invertebrates

Regulation of ion flux

Like other freshwater bivalves, *Ligumia subrostrata* maintains its body fluids hyperosmotic to the aquatic medium, largely by regulating the flux of sodium, its major blood cation (Dietz, 1977, 1979). PGE_2 appears to be a component of the sodium regulation system because inhibition of endogenous PG-biosynthesis by injection of indomethacin, a potent cyclooxygenase inhibitor in mammals, increased sodium flux. The effect lasted about 15 hours, approximately doubling the control values. Alternatively, when PGE_2 was injected in parallel experiments, sodium influx declined about 5-fold from control values. Since chloride concentrations and sodium outflux remained unchanged during these experiments, Graves and Dietz (1979) concluded that PGE_2 participates in ion regulation by specifically controlling sodium influx. A tissue specificity may also exist because indomethacin modified the activity of the epithelial cells involved in sodium uptake without changing urinary sodium loss.

Indomethacin stimulated sodium influx in a dose-dependent way over the concentration range of 0.05 to 0.25 μmol/g dry wt. Other PG-synthetase inhibitors in mammals—meclofenamate (a cyclooxygenase inhibitor), and dexamethasone (which inhibits phospholipase A_2, and hence, regulates substrate availability)—also stimulate sodium uptake (Saintsing and Dietz, 1983). The stimulatory effect of PG-synthetase inhibitors was neutralized by co-injection of PGE_2, supporting the view that PG is part of the system regulating epithelial sodium flux. PGE_2 reduces influx; reduction of PGE_2 biosynthesis may increase influx by attenuating the PG inhibition of uptake, but positive stimulation seems to depend on a biogenic amine, 5-hydroxytryptamine (5-HT, or serotonin), rather than on another PG since $PGF_{2\alpha}$ acts much like PGE_2 (Saintsing and Dietz, 1983). Cyclic AMP (cAMP) also stimulates sodium uptake (Graves and Dietz, 1982), which suggests that PG inhibition and 5-HT stimulation of sodium flux may both function via antagonistic effects on adenyl cyclase activity.

Arachidonic acid injections apparently increased renal outflux of sodium without changing epithelial uptake. Graves and Dietz (1979) suggested that the arachidonic acid may initially alter renal function, and be metabolized too quickly to allow formation of inhibitory levels of PGE_2 in epithelial tissue. Another possibility is that ion regulation is more complex (Graves and Dietz, 1982). If, as in mammals, arachidonic acid is potentially metabolized into a variety of prostanoid compounds, then we can imagine one metabolite, PGE_2, inhibiting epithelial uptake while others, not yet identified, modify renal ion flux in ways still unknown.

The idea that PGs regulate epithelial sodium uptake in a freshwater mussel is based mostly on pharmacological treatments with appropriate compounds. Saintsing *et al.* (1983) showed the presence of PGs in *L. subrostrata* extracts by RIA, lending further support to natural occurrence and biological activity in an aquatic invertebrate.

PGE_2 is also involved in ion regulation in the marine bivalve *Modiolus demissus* (Freas and Grollman, 1980). When isolated gills were subjected to hypoosmotic stress by incubation for 60 minutes in 25% seawater, there was a 10-fold increase in PGE_2 released into the medium, suggesting an increase in biosynthesis and release of the

PG. In addition to this osmotic action on PG release, there is a specific ionic effect. To test for possible ionic effects, gills were incubated in artificial seawater of fixed osmotic concentration, but selectively free of sodium, calcium, potassium, or magnesium. Only the magnesium-free artificial seawater stimulated gills to increase PGE_2 release. However, the apparent osmotic effect is not due solely to depletion of environmental magnesium because gills incubated in hypoosmotic seawater with normal magnesium concentrations also induced increased PG release. Hence, gill tissues of this marine bivalve respond to changes in osmotic and ionic concentrations.

In mammals, the physiological activities of many PGs are mediated by specific cellular receptor sites. Freas and Grollman (1981) showed the existence of specific PGA_2 binding sites in homogenates of gills, mantles, siphons, adductor, and upper and lower visceral masses. In gills, these sites were ionic, pH dependent, and reversible. To date this is the only study of PG binding sites in invertebrate tissues; such a finding adds considerable verisimilitude to physiological propeties of PGs.

Mediation of behavioral thermoregulation and fever

PGE_1 appears to mediate febrile response to infection in a number of mammals, including monkeys (Crawshaw and Still, 1975), sheep (Hales *et al.,* 1973), rabbits (Stitt, 1973; Lin, 1978), cats (Milton and Wendlandt, 1970; 1971), and guinea pigs (Szekely and Komaroni, 1978). Fever also occurs in non-mammalian vertebrates, although the increased body temperatures appear to be mediated by behavioral as opposed to endogeneous physiological mechanisms. Behavioral fever has been observed in frogs (Casterlin and Reynolds, 1977a, Myhre *et al.,* 1977), a lizard (Bernheim and Kluger, 1976), and several fishes (Reynolds *et al.,* 1976).

Some aquatic invertebrates express behavioral fever in response to bacterial infection by moving into a zone of warmer water. The freshwater crayfish *Cambarus bartoni* exhibited a 2°C behavioral fever after innoculation with a suspension of killed bacteria (*Aeromonas hydrophila*) by choosing higher temperatures in a gradient trough (Casterlin and Reynolds, 1977b). This behavioral response to infection may be mediated by endogenous formation of PGE_1 because increasing doses of the PG also induced 1 to 3.5°C fevers when injected over the range of 50 to 500 μg/individual (Casterlin and Reynolds, 1978). Three marine arthropods—the American lobster *Homarus americanus,* the pink shrimp *Penaeus duorarum,* and the horseshoe crab *Limulus polyphemus*—similarly increased their temperature preferenda by more than 4°C in response to 100 μg injections of PGE_1 (Casterlin and Reynolds, 1979).

Two terrestrial arthropods, the scorpions *Bathus occitanus* and *Androctonnus australis,* regulated their body temperatures by selecting appropriate positions along temperature gradients in a sand box. *A. australis* increased temperature preferences by 15°C and *B. occitanus* by 20°C after treatment with physiological doses of PGE_1 (Cabanac and Le Guelte, 1980). Although it is not known whether these species generate fever due to bacterial infection, it appears that PGs may be involved in some aspect of behavioral thermoregulation.

Together, these reports suggest that PGs may be some part of the thermoregulatory physiology of many invertebrates. The idea is based on the observation of increased body temperatures in response to individual doses of a single compound, namely PGE_1. Important detailed biochemical questions remain unanswered: do PGs naturally occur in these species? Does PG biosynthesis increase after infection, but before the febrile response? Do all PGs induce fever, or is a more specific set of these compounds involved? Research in this area may assume ecological interest, as suggested by remarks below.

Among terrestrial invertebrates, many medium to large size insects regulate thoracic temperatures to a set point suited to the high metabolic demands of powered flight by behavioral (Casey, 1981) or physiological (Kammer, 1981) means. In addition to flying insects, thermoregulation has been studied in caterpillers of two sphinx moths, *Hyles lineata* and *Manduca sexta* (Casey, 1976, 1977). *H. lineata* appears to sustain high body temperatures and correspondingly high rates of feeding by basking in appropriate postures; *M. sexta* does not maintain high temperatures even though feeding and growth rates are reduced considerably at cooler temperatures. These different behaviors appear to be linked to differences in predator defense mechanisms and in seasonal availability of their host plants. Other caterpillers, including the butterflies *Vanessa io* and *V. urtica,* huddle in groups, resulting in increased body temperature and development rates (Mosebach-Pukowski, 1938). Similarly, the larvae of wax moths thermoregulate, partly, by huddling or scattering (Smith, 1941).

Many insect species are resistant to viral infection when maintained at higher temperatures (Tanada, 1967). Watanabe and Tanada (1972) reviewed several lepidopteran cases of insect viruses which do not cause lethal infections at higher temperatures, including larvae of the armyworm *Pseudaletia unipunctata,* the cabbage looper *Trichoplusia ni,* and the corn ear worm *Heliothis zea.* Hence, behavioral thermoregulation in invertebrates may effect such biological parameters as feeding and development rates, and resistance to disease. PGs may be an important biochemical mediator in this area of physiological ecology.

Control of hatching

In the barnacle *Balanus balanoides,* full egg-laying involves passing eggs along oviducts into ovisacs produced by oviducal glands. Fully formed egg masses are finally released into the mantle cavity, where they remain until hatching which corresponds with spring algal blooms (Crisp, 1962; Clare *et al.,* 1985). The synchrony of spring bloom and egg hatching could be related to a component in the nutrition of adult barnacles. However, Crisp and Spenser (1958) showed that seawater extracts of unfed and fed adults were equally effective in inducing hatching. They proposed a barnacle hatching substance, endogenously produced by adults, and showed that the substance acts upon the musculature of mature embryos, not on the egg case.

The hatching substance appeared to be a PG (Clare *et al.,* 1982, 1985). The substance is extractable in a system optimized for PGs, it behaves like a PG on thin layer chromatography, and extracts of the dried cortex of a commercial source of PG (the gorgonian *Plexura homomalla*) acted biologically and chemically like barnacle hatching substance. Extracts made in the presence of aspirin—a PG-synthetase inhibitor in mammals—did not induce hatching. Clare *et al.* (1985) concluded that barnacle hatching substance is either a PG or a PG-like compound.

Subsequent work underscores the importance of rigorous chemical methodologies in indentification of biologically active compounds. Holland *et al.* (1985) extracted 50 kg of barnacles, then processed the extracts through four sequential systems of thin layer chromatography. The active compound was detected by bioassay at each stage. The purified compound was derivatized for gas chromatography-mass spectroscopy (GC-MS), which yielded a single major GC peak. Mass spectra of derivatized hatching factor and hydrogenated derivatized hatching factor were consistent, not with a PG, but with another eicosanoid, 10,11,12-trihydroxy-5,8,11,17 eicosatetraenoic acid (Fig. 5). This compound is probably a lipoxygenase derivative of C20:5n3, an abundantly available fatty acid in marine invertebrates and also the precursor of the 3-series PGs.

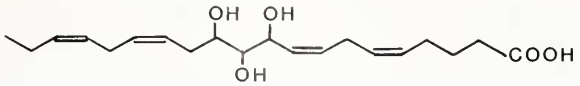

FIGURE 5. Structure of barnacle hatching factor, 10,11,12-trihydroxy-5,8,11,17-eicosatetraenoic acid.

Reproduction in Mollusca

PGs appear to stimulate egg production in the freshwater snail *Helisoma durgi* (Kunigelis and Saleuddin, 1986). When injected directly into the haemocoel of adults, ng quantities of PGE_2 produced apparent discomfort in all individuals and even death in isolated cases with no increase in egg masses or in eggs per mass. But when introduced into the female genital opening in a viscous fluid designed to approximate semem, PGE_2 treatments stimulated a long-term increase in egg production. Four weeks after treatment of virgin snails with 25, 50, and 100 ng doses of PGE_2, cumulative egg production was about 200, 425, and 650 eggs per animal, respectively.

Reproductive tissues from virgin and mated snails, the ovotestis, seminal vesicle, bursa copulatrix, and oothecal gland presented substantial PG-biosynthetic activity *in vitro*. Mating significantly altered the activity in two of the tissues. In ovotestis, synthesis of PGE_2 decreased while PGA_2 synthesis increased with no change in synthesis of $PGF_{2\alpha}$. Synthetic activity changed in the bursa copulatrix, with PGE_2 and PGA_2 reduced to effective zero after mating; $PGF_{2\alpha}$ was again unchanged. Differences in PG-synthetic activity did not occur in seminal vesicle or oothecal gland (Kunigelis and Saleuddin, 1986). These two lines of evidence—the effects of PG treatments on egg production and alterations in PG-synthetic activity—suggest that PGs play important reproductive roles in this snail.

PGs are also produced by accessory sex glands of another snail, *Lymnaea stagnalis* (Clare *et al.*, 1986). Homogenates of the albumen gland, bursa copulatrix, prostrate gland, and seminal vesicles converted radioactive arachidonic acid into labelled products that co-eluted with 6-keto-PGE_1, PGE_2, PGA_2/B_2 (not resolved), thromboxane B_2 (TxB_2), and several unknown compounds. Whole organs also converted arachidonic acid into these compounds, although in proportions different from the homogenates of the same organs. Effects of mating on PG-biosynthetic activity were not tested, nor were effects of PG administration on reproductive functions; nonetheless, the PGs formed in the reproductive organs eventually may be shown to play a still undefined role.

PGs induce spawning in two other molluscs, the abalone *Haliotis refescens* and the mussel *Mytilus californianus*. When added to seawater cultures at 3×10^{-12} *M*, PGE induced about a third and PGF about a half of male and female abalone to spawn (Morse *et al.*, 1977). Although the physiological mechanisms remain unclear, important biochemical insights have emerged. Addition of hydrogen peroxide to seawater tanks induced synchronous spawning in *H. refescens* and *M. californianus*. This observation is connected to the biochemistry of PG biosynthesis as understood in mammals. The first step in the conversion of arachidonic acid to the 2-series PGs is catalyzed by fatty acid cyclooxygenase (also known as prostaglandin endoperoxide synthetase). This involves first activation of the enzyme by a hydroperoxy group, then elimination of a hydrogen atom from C13 of arachidonic acid, leaving a free radical. This is followed by adding a peroxy radical in a bridge across C9 and C11, formation of the 8,12 carbon-carbon bond (required for the cyclopentane ring in the final prod-

uct), isomerization of the 11,12 double bond to 12,13, and addition of another peroxy radical to C15, with concomitant isomerization of the 12,13 double bond to 13,14. These final electron shifts generate PGG_2, a short-lived intermediate in the conversion of arachidonic acid to PG. The hydrogen peroxide effect is pH dependent, with lower concentrations releasing spawning at higher alkalinity. Morse *et al.* (1977) suggested that the alkaline conditions (pH 9.1) favored decomposition of hydrogen peroxide to the highly reactive hydroperoxy free radical. Since a hydroperoxy group activates the enzyme and peroxy radicals are added in two steps in the formation of PGG_2, the free radicals derived from hydrogen peroxide may enhance overall conversion of precursor fatty acids to PGs.

PGs appear to be important in basic physiological functions in molluscs, including ion regulation, possible renal function, and reproductive biology. This preliminary work sets the stage for important questions of the precise physiological activity, and offers the possibility of gaining greater understanding of invertebrate physiology and appreciation of PGs in these systems.

Oocyte maturation in starfish

Starfish oocytes develop to the first meiotic prophase, then await the spawning period. Maturation, or meiosis reinitiation, is induced by a hormone produced and released by the follicle cells surrounding the oocytes, 1-methyladenine. Once stimulated by the hormone, the oocytes complete the developmental path leading to fertilizable cells.

Arachidonic and eicosapentaenoic acids also induce oocyte maturation in three species of starfish: *Asterias rubens, Marthosterius glacialis,* and *Luidia ciliaris* (Meijer *et al.,* 1984). The PUFA-induced maturation is specific to these two fatty acids because 35 other fatty acids, ranging from C4:0 to C24:1 and including saturated, monounsaturated, and polyunsaturated fatty acids, did not induce maturation. The maturation effect is dependent upon extracellular calcium and occurs at physiological concentrations (*i.e.,* 50% maturation dose = 0.65 μM arachidonic acid). The fatty acids stimulate the complete maturation program, including germinal vesicle breakdown, fertilization, and development into normal larvae. Fatty acids endogenous to the oocytes are able to stimulate maturation because addition of phospholipase A_2, an enzyme that hydrolyses the fatty acid from the beta-carbon of phospholipids, also stimulated maturation. The phospholipase effect was calcium-dependent, and specific because phospholipases C and D did not bring on maturation.

The hormone effect probably proceeds through release and metabolism of PUFAs. Two phospholipase A_2 inhibitors in mammals, quinacrine and bromophenacyl bromide, inhibit hormone-stimulated maturation, which can be overcome by increasing 1-methyl adenine concentrations. Five PGs did not stimulate maturation, and three cyclooxygenase inhibitors—acetylsalicylic acid, indomethacin, and tolazoline—did not inhibit maturation. On the other hand, three lipoxygenase inhibitors in mammals—quercetin, eicosatetraynoic acid and butylated hydroxytoluene—did inhibit hormone-induced maturation. Four products of lipoxygenase metabolism of arachidonic acid, 12- and 15-hydroxyeicosatetranoic acids (HETE) and their corresponding hydroperoxyeicosatetraenoic acids (HPETE) stimulated maturation.

Oocytes convert radioactive arachidonic acid into HETEs (Meijer *et al.,* 1986a). Conversion of arachidonic acid does not occur in the absence of calcium, nor are oocytes stimulated to maturation. Following incubation with radioactive arachidonic acid, fractions with chromatographic behavior of HETEs were recovered and found to stimulate oocyte maturation. The lipoxygenase inhibitor eicosatetraynoic acid in-

hibited both conversion of arachidonic acid and stimulation of oocytes. It would appear, then, that 1-methyladenine acts by release of PUFA, followed by conversion to a biologically active HETE, which induces maturation of the oocytes.

Injection studies suggested that 12- and 15-HETE and corresponding HPETEs stimulated oocyte maturation (Meijer *et al.*, 1984). Upon re-evaluation, it was found that the tested compounds were contaminated with 5% of 8-HETE, the active compound in maturation (Meijer *et al.*, 1986a). Meijer *et al.* (1987) showed that (8R)-HETE, but not (8S)-HETE, is produced by starfish oocytes. The R isomer is the only active compound when tested in pure form, and other lipoxygenase products, including other HETEs and leukotrienes are not active.

A survey of eight starfish species shows that while the hormone 1-methyladenine stimulates maturation in all species, the stimulatory effect of arachidonic acid and 8-HETE occurs in only three of them (Meijer *et al.*, 1986b). Species differences in response to various eicosanoids also have been observed in various physiological settings in mammals. At this early period of appreciating the possible physiological activities of these compounds in invertebrates systems, species differences underscore the hazards inherent in forming generalizations.

Cercarial penetration of skin

Eggs of the blood fluke *Schistosoma mansoni* leave their mammalian hosts in urine or feces, and continue larval development in snails. Free-swimming larvae called cercariae reinfect mammalian hosts by burrowing through the skin or by ingestion with drinking water (Storer and Usinger, 1965). It has been known for a number of years that skin surface lipids stimulate cercarial penetration of animal membranes (Stirewalt, 1971). Among the skin surface lipids, free fatty acids, especially polyunsaturated fatty acids, appeared to be most efficacious in stimulating penetration (Austin *et al.*, 1972). Salafsky *et al.* (1984a) looked at the effect of certain fatty acids on two cercarial behaviors *in vitro*, namely cessation of swimming and initiation of penetration. Their results show that certain PUFAs attracted cercariae to the center of their test membranes while monounsaturated fatty acids did not. A few fatty acids gave intermediate results because two monounsaturated fatty acids were as stimulatory as the PUFAs, and two other monounsaturates were less stimulatory than the PUFAs but were clearly more stimulatory than controls. Cyclooxygenase metabolites, rather than the PUFAs *per se*, may alter cercarial behavior. Two inhibitors of cyclooxygenase—ibuprofen and, to a lesser degree, aspirin—inhibited cercarial response to PUFA. 13-Azaprostanoic acid, thought to specifically antagonize the platelet thromboxane/endoperoxide receptor in mammals, was also inhibitory.

PUFAs and certain of their metabolites may affect cercarial penetration as well as modify behaviors that precede penetration. When Salafsky *et al.* (1984b) compared cercarial penetration into skin membranes prepared from essential fatty acid (EFA) deficient and EFA replete adult rats, they found about three times less penetration in the preparations from EFA deficient rats. Again, the inhibition may be related to formation of eicosanoids. Interperitoneal injections of ibuprofen led to a time-dependent accumulation of the drug in the skin of EFA replete rats. Cercarial penetration of the drug-treated skin was reduced. The percent inhibition increased with increasing amount of ibuprofen accumulated in the skin, up to a maximum inhibition of about 84%.

When cercariae were incubated with radioactive linoleic acid, radioactivity could be recovered in high-pressure liquid chromatography fractions that eluted with PGE_2, PGD_2, LTC_4, LTB_4 and 5-HETE. These data suggest that cyclooxygenase and

lipoxygenase systems function within the cercariae. Radioimmunoassays of extracts from cercariae incubated with linoleic acid were also consistent with these products. Fusco *et al.* (1985) concluded that formation of eicosanoids is an essential step in penetration of human skin by cercariae of *Schistosoma mansoni*. If this can be supported by further work, it may present a rather interesting situation in which the PUFA substrate provided by a vertebrate host is metabolized into biologically active eicosanoids by a parasite.

It is not yet known how the eicosanoids alter the behavior of the cercariae or facilitate penetration of mammalian skin. Fusco *et al.* (1985) suggest that vasodilation, which is induced by certain PGs, may help the parasite find and infiltrate the blood system. It would appear that the eicosanoids, in this mode, would be usurped by the parasites to alter the host physiology. In this case, the finding by Rumjanek and Simpson (1980) that adult worms do not synthesize PGE or PGF may be appreciated in terms of host physiology. On the other hand, the behavioral effects of cessation of swimming and initiation of penetration (Salafsky *et al.*, 1984a), also induced by skin lipids, suggest a direct effect on the cercariae.

Sponge cell aggregation

Rich *et al.* (1984) suggest that the calcium dependent aggregation of marine sponge cells of *Microcione prolifera* is stimulated by leukotriene B_4 (LTB_4). LTB_4 induced rapid cell aggregation in a dose-dependent way at 0.2 and 1.2 μM treatments. The effect appears to be specific for LTB_4 because eight PGs of A, B, D, E, and F series and eight lipoxygenase products failed to induce aggregation.

The calcium ionophore A23187 and the species-specific aggregation factor (MAF) stimulate cell aggregation. The aggregating effects of these compounds can be inhibited by cyclooxygenase inhibitors including nordihydroquaiaretic acid and indomethicin, which also interfere with calcium flux. These data show that those agents which inhibit calcium flux also inhibit aggregation while those that promote calcium movement also promote aggregation. Interpretation is difficult because while a specific lipoxygenase product promotes aggregation, inhibitors of cyclooxygenase metabolism inhibit it. Perhaps both pathways are involved in cell aggregation, with LTB_4 stimulating PG formation, which then acts in concert with the LTB_4.

Egg-laying behavior in crickets

The roles of PGs in insect reproduction were reviewed by Stanley-Samuelson and Loher (1986), from which the following summary is drawn. PGs were detected in extracts of various tissues from over a dozen species of insects. The most well understood physiological role of PGs is releasing egg-laying behavior in the field cricket *Teleogryllus commodus*. Adult females undergo sexual maturation, during which the abdomen becomes filled with hundreds of mature eggs. Certain behaviors that are likely to bring females into contact with males also develop. Insemination is achieved by transfer of a spermatophore to the genital organ of a female from where its contents migrate into the female's spermathecae. Cyclooxygenase activity is associated with the spermatophore contents, and once in the spermathecae of newly mated females, arachidonic acid is converted into PG.

It is not known how the PG formed in the spermatheca releases egg-laying behavior, but increases in spermathecal and hemolymph PG titer after mating suggest that the PG acts at some site distant from the source. The observations that PGE_2 does not stimulate contraction of oviduct muscles in *T. commodus* (Loher, 1984) nor in a

cockroach (Cook *et al.*, 1984) and that oviposition behavior is a complex activity directed by the central, rather than peripheral, nervous system (Loher, 1984) support the hypothesis that the PGs function at the level of the central nervous system.

Using egg-laying to assay structure-function relationships among a range of eicosanoids, Stanley-Samuelson *et al.* (1986) found that highest egg-laying activity was associated with E-series PGs. The A-, B-, D- and F-series induced zero to intermediate egg-laying. Structures that departed from the basic PG structure, represented by 15-HETE and prostacyclin, were inactive. The 2-series PGs were more active than their 1-series analogues; hence, there may be a biological specificity for PGE_2 in releasing egg-laying behavior in that particular cricket species.

Highest egg-laying activity was induced by 15-keto-PGE_2. In mammalian systems, this compound is formed by the action of prostaglandin dehydrogenase, located mainly in lungs, but also in liver and kidney. Biologically active PGE is rapidly cleared from the circulation of mammals by the activity of this enzyme. The observation that a biologically inactive compound, in the usual mammal assays, was associated with the greatest increase in egg-laying behavior marks a potentially important point in comparative physiology. Several features of the biology of eicosanoids appear to uniformly occur in the vertebrate and invertebrate systems as understood to date. For example, many compounds that inhibit the action of cyclooxygenase in mammals similarly inhibit the activity in invertebrates. On the other hand, as shown here, while the mammalian background will be important and useful in work on invertebrate systems, fundamental differences are to be expected.

Perspectives

Various eicosanoids appear to be involved in the regulation of a variety of physiological and behavioral areas in representatives of many invertebrate phyla. In some cases (such as mediation of behavioral thermoregulation), the evidence for an eicosanoid function is based on treatment of animals with a single compound and observation of the response. At this level of observation, it remains to be established that eicosanoids are physiologically involved. Given a good base of preliminary observations, important research goals would be to firmly show that, in the case at hand, PGs do mediate thermoregulatory behavior. In still other cases, such as the role of PG in releasing egg-laying behavior in crickets, there is sufficient evidence to accept that certain PGs do release egg-laying, although some details of the physiological mechanism—where in the central nervous system PGs act and how they alter behavior—are not yet understood. Research in this area could usefully be aimed, not at reaffirming the role of the eicosanoid, but at aquiring more details of the action. In study areas where considerable biochemical details are established—as in starfish oocyte maturation—cellular events remain unknown. Again, understanding how eicosanoids act remains a major research goal.

We are aware of eicosanoid roles in particular physiological areas in a given invertebrate organism. We know, for example, that PG releases egg-laying behavior in females of the cricket *T. commodus*. PGs are also detected in salivary glands, endocrine glands, Malpighian tubules, testes, and ventral nerve cords. Aside from the known role in altering behavior, what do PGs contribute to the other tissue systems in which they appear? Are they involved in regulating ion flux in Malpighian tubules, secretion in salivary glands, and neural function in the nerve cord, within the same organism?

Eicosanoids appear to be produced and to act at local, tissue, or cellular levels in mammals. PGE_1 produced by adipocytes functions within the same cells to modulate

the lipid mobilizing effect of certain hormones. Moreover, there are mechanisms that block PG circulation. The global circulation of PGE_2, for example, is checked by the action of prostaglandin dehydrogenase, located mainly in lungs, which converts the active compound into a biologically inactive product. However, in the cricket *T. commodus*, it is speculated that the release of egg-laying behavior by PGE_2 is mediated in a manner more like a broadly circulating hormone (Stanley-Samuelson and Loher, 1986; Stanley-Samuelson *et al.*, 1986). This point can be extended to research in the mode of action, at the whole-organism level, of eicosanoids in invertebrates. The trihydroxyeicosatetraenoic acid that functions as hatching substance in the barnacle may be an example of a compound produced in one organ system with its action observed elsewhere, again suggesting hormone action.

With several likely roles of eicosanoids set forth, general research areas include establishing more firmly the activities, elucidating cellular details, and appreciating the possible modes of action of these compounds. One can assume that as details of eicosanoid action become known they will contribute greatly to our understanding of invertebrate physiology.

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